Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 11460–11465, October 1996
Applied Biological Sciences

Whole body nitric oxide synthesis in healthy men determined from [^{15}N]arginine-to-[^{15}N]citrulline labeling

LETICIA CASTILLO*†, LOUIS BEAUMIER*, ALFRED M. AJAMI‡, AND VERNON R. YOUNG*†

*Laboratory of Human Nutrition and Clinical Research Center, Massachusetts Institute of Technology, Cambridge, MA 02139; †Shriners Burns Institute and Massachusetts General Hospital, Boston, MA 02114; and ‡Mass Trace, Inc., Woburn, MA 01801

Contributed by Vernon R. Young, July 12, 1996

ABSTRACT **The rates of whole body nitric oxide (NO) synthesis, plasma arginine flux, and *de novo* arginine synthesis and their relationships to urea production, were examined in a total of seven healthy adults receiving an L-amino acid diet for 6 days. NO synthesis was estimated by the rate of conversion of the [^{15}N]guanidino nitrogen of arginine to plasma [^{15}N]ureido citrulline and compared with that based on urinary nitrite (NO_2^-)/nitrate (NO_3^-) excretion. Six subjects received on dietary day 7, a 24-hr (12-hr fed/12-hr fasted) primed, constant, intravenous infusion of L-[*guanidino*-$^{15}N_2$]arginine and [^{13}C]urea. A similar investigation was repeated with three of these subjects, plus an additional subject, in which they received L-[*ureido*-^{13}C]citrulline, to determine plasma citrulline fluxes. The estimated rates (mean ± SD) of NO synthesis over a period of 24 hr averaged 0.96 ± 0.1 μmol·kg^{-1}·hr^{-1} and 0.95 ± 0.1 μmol·kg^{-1}·hr^{-1}, for the [^{15}N]citrulline and the nitrite/nitrate methods, respectively. About 15% of the plasma arginine turnover was associated with urea formation and 1.2% with NO formation. *De novo* arginine synthesis averaged 9.2 ± 1.4 μmol·kg^{-1}·hr^{-1}, indicating that ≈11% of the plasma arginine flux originates via conversion of plasma citrulline to arginine. Thus, the fraction of the plasma arginine flux associated with NO and also urea synthesis in healthy humans is small, although the plasma arginine compartment serves as a significant precursor pool (54%) for whole body NO formation. This tracer model should be useful for exploring these metabolic relationships *in vivo*, under specific pathophysiologic states where the L-arginine–NO pathway might be altered.**

Arginine serves various important metabolic functions, including roles in protein synthesis, nitrogen transport and elimination (1, 2), and as the precursor of nitric oxide (NO), which is a current area of considerable research interest (3–5). Oxidation of arginine by a homodimeric group of nitric oxide synthases yields citrulline and nitric oxide in stoichiometric amounts, with each containing a nitrogen atom derived from the guanidino moiety of arginine. NO undergoes oxidative degradation to the stable end products nitrite (NO_2^-) and nitrate (NO_3^-). In the *in vivo* system, NO_2^- is readily oxidized to NO_3^- via hemoglobin, and so NO is eventually detected in plasma or urine as NO_3^- (5) and its measurement has been used to estimate the rate of whole body NO synthesis.

It has been suggested that therapeutic modulation of nitric oxide production may be achieved by supplying the precursor arginine (6–9) or by inhibiting NO production with L-arginine analogs (10–12). However, before profound changes in exogenous arginine intake levels or a pharmacologic inhibition of NO formation can be safely recommended, it is desirable to gain a better understanding of the regulation of whole body arginine metabolism in human subjects and its quantitative interrelationships with the L-arginine–NO pathway.

In our previous studies, using stable isotopic tracer techniques, we have investigated whole body arginine metabolism and the use of arginine for NO synthesis, by measurement of the transfer of the guanidino nitrogen of plasma arginine to urinary NO_3^-. These studies have been carried out in healthy humans receiving variable levels of arginine intake (13–15), and in infants with pulmonary hypertension (16). We have now extended these earlier investigations of whole body arginine homeostasis and the metabolism of urea cycle intermediates by examining the kinetics of arginine metabolism throughout a continuous 24–35-hr period. Using this design, it has been possible, for the first time to our knowledge, to determine the *in vivo* rate of conversion of [^{15}N]guanidino arginine to [^{15}N]ureido citrulline and NO, presumably due to the NO synthase reaction. We compared this estimate with the rate of transfer of the guanidino nitrogen of arginine to urinary nitrite (NO_2^-)/nitrate (NO_3^-). Simultaneously, we have been able to examine the rate of whole body *de novo* arginine synthesis and the rate of transfer of plasma arginine to urea over a 24-hr period, in healthy subjects receiving an adequate arginine intake. This investigation establishes an experimental basis for the design and conduct of follow-up studies intended to explore the consequences of altered arginine intakes or availability on NO synthesis in humans under various pathophysiological states.

MATERIALS AND METHODS

Subjects. A total of seven healthy young adult males (age 23 ± 3 years; weight 74 ± 6.5 kg) participated in this investigation, which was conducted at the Clinical Research Center (CRC) of the Massachusetts Institute of Technology (MIT). All were in good health, as established by medical history, physical examination, analysis of blood cell count, routine blood biochemical profile, and urine analysis. Subjects were fully informed about the purpose of the study and the potential risks involved. Written consent was obtained according to the protocol approved by the MIT Committee on the Use of Humans as Experimental Subjects and the Advisory Committee of the CRC. All subjects received financial compensation. Six subjects participated in the 35-hr-labeled arginine/urea tracer experiment. Also, a second study with four subjects was conducted for determination of 24-hr plasma citrulline fluxes and *de novo* arginine synthesis. Three of these subjects had participated in the first study.

Diet and Experimental Design. Individual daily energy intake was calculated to maintain body weight, and in both studies subjects consumed for 6 days a low nitrate, complete L-amino acid diet patterned after egg protein (13), supplying the equivalent of 1 g of protein (N × 6.25) per kg of body weight per day. The diet also supplied an average daily nitrate intake of 210 μmol. Further details concerning diets and procedures have been described elsewhere (13, 17).

Urine Collections. Complete 24 hr urine collections were obtained in brown plastic bottles containing 15 ml (6 M) HCl.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Tracer Infusion Studies. Following an overnight fast, on the morning of day 7, subjects participating in the 35-hr study received priming doses of L-[*guanidino*-$^{15}N_2$]arginine ([$^{15}N_2$]arginine; 5 μmol·kg^{-1}) and [^{13}C]urea (170 μmol·kg^{-1}) over 3 min and these were followed by constant intravenous infusions of [$^{15}N_2$]arginine (5 μmol·kg^{-1}·hr^{-1}) and [^{13}C]urea (8 μmol·kg^{-1}·hr^{-1}) for 24 hr. Tracers (>98 atom percent excess) were purchased from Mass Trace (Woburn, MA), and infusates prepared from sterile, pyrogen-free powders of high chemical purity. During the first 12 hr, subjects received their total protein and energy intake as small, isonitrogenous meals given every hour for the first 12 hr (0–720 min). During the following 12 hr (780–1440 min) they remained in the post-absorptive or fasting state.

Blood samples were obtained at baseline and at frequent intervals throughout the 24-hr period. Consecutive, 3-hr urine collections were obtained during the 24-hr tracer period and also for the 11-hr post-tracer period, to determine the "decay" in the plasma isotopic enrichment of the tracers and in urinary [^{15}N]nitrate (Fig. 1). The subjects received their amino acid diet in three meals, during the 11-hr urine collection period. Other details related to sample collection and storage are given elsewhere (13).

For the second tracer study, the dietary adaptation period and procedures were identical to those described above for the 35-hr study. A prime (1 μmol·kg^{-1}), followed by a constant tracer dose of L-[*ureido*-^{13}C]citrulline (1 μmol·kg^{-1}·hr^{-1}), was given to four subjects who were in the fed state for the initial 12 hr and in the post-absorptive or fasting state for the consecutive 12 hr. Blood samples were obtained at frequent intervals, but urine collections were not performed in this case.

Analysis. Determination of plasma isotopic abundance in [$^{15}N_2$]arginine and [^{13}C]citrulline infused and of the [^{15}N]citrulline and [^{13}C]arginine formed *in vivo* was performed, using 200 μl of plasma, as described (13–15). Tracer mole percent excess (MPE) was calculated by statistical mass isotopomer analysis (18), because stock solutions of citrulline isotopologs proved to be too unstable for repeated application in the construction of sufficiently accurate and precise standard curves. In method validation experiments for this study, the improved analytical approach afforded an average accuracy error of less than 5% and an intersample precision of less than 7% for determinations of [^{15}N]citrulline in the 0.05 to 1 MPE range. We were not able to detect mono-^{15}N-labeled arginine, which would have been derived from [^{15}N]citrulline. Isotopic abundance in plasma [^{13}C]urea and of [$^{15}N_2$]urea, derived from the guanidino-labeled arginine, was measured as described (19, 20). Measurements of isotopic abundance of [^{15}N]nitrate can be affected by the pH of the preserved sample. Therefore, we evaluated the effects of collecting urine samples in 6 M HCl. A sample of fresh urine was divided in two aliquots and collected in acid with 6 M HCl or in 6 M NaOH. The two aliquots were subdivided and a known amount of [^{15}N]nitrate was added to increase the natural isotopic abundance from 0 to 15% APE. Each sample was analyzed for [^{15}N]nitrate as described (21) and compared with expected values. Measured isotopic abundance of [^{15}N]nitrate in the acidified group was lower than their expected values, but measurements carried out in those samples collected in alkaline solution were close to predicted values. The differences between the two methods of collection was constant (78%), within the range of enrichments evaluated. Therefore, all reported measurements of the isotopic abundance of [^{15}N]nitrate were corrected for the underestimate of 22%, related to the conditions used in sample collection. Deproteinized urine samples were used to determine total nitrite/nitrate concentrations, by the Griess reaction (21). Plasma and urine urea nitrogen concentrations also were measured (22).

***In Vivo* Model of Amino Acid Kinetics.** Plasma arginine and citrulline fluxes were calculated from the mean plasma isotopic enrichment values for the last 6 hr of both fed and fasted periods, using steady-state isotope dilution equation as described (14). The rates of conversion of [$^{15}N_2$]arginine to [^{15}N]citrulline and to [$^{15}N_2$]urea ($Q_{Arg\to Cit}$; $Q_{Arg\to urea}$) (Study 1) and that of [*ureido*-^{13}C]citrulline to [^{13}C]arginine ($Q_{Cit\to Arg}$) (Study 2), were determined essentially according to the tracer model of Clarke and Bier (23) and Thompson *et al.* (24), which they had used earlier for measurement of conversion of labeled phenylalanine to tyrosine, and as we have described in previous reports (14, 15, 25). For the three subjects in the [^{15}N]arginine tracer study and in whom citrulline fluxes were not measured, we used for calculation purposes the mean of the citrulline fluxes, for the fasted and fed states, obtained from the four subjects in which these determinations were made directly. For estimation of the rate of conversion of citrulline to arginine in the four subjects given labeled citrulline we measured the ^{13}C abundance of arginine and applied the previously measured arginine fluxes for the three repeat subjects and the mean of the arginine fluxes determined in the group of six subjects as an estimate of the arginine flux for the fourth subject in the [*ureido*-^{13}C]citrulline group.

Briefly, calculation of the rate of conversion of arginine to citrulline, via the NO synthesis reaction, was made as follows:

$$Q_{Arg\to Cit} = Q_{Cit} \times \frac{E_{Cit}}{E_{Arg}} \times \left[\frac{Q_{Arg}}{I_{Arg} + Q_{Arg}}\right],$$

where Q_{Arg} and Q_{Cit} are the plasma arginine and citrulline fluxes (μmol·kg^{-1}·hr^{-1}), respectively, estimated from the primed, constant infusions of the L-[*guanidino*-$^{15}N_2$]arginine and L-[*ureido*-^{13}C]citrulline tracers; E_{Cit} and E_{Arg} are the respective plasma enrichments of [*ureido*-^{15}N]citrulline and

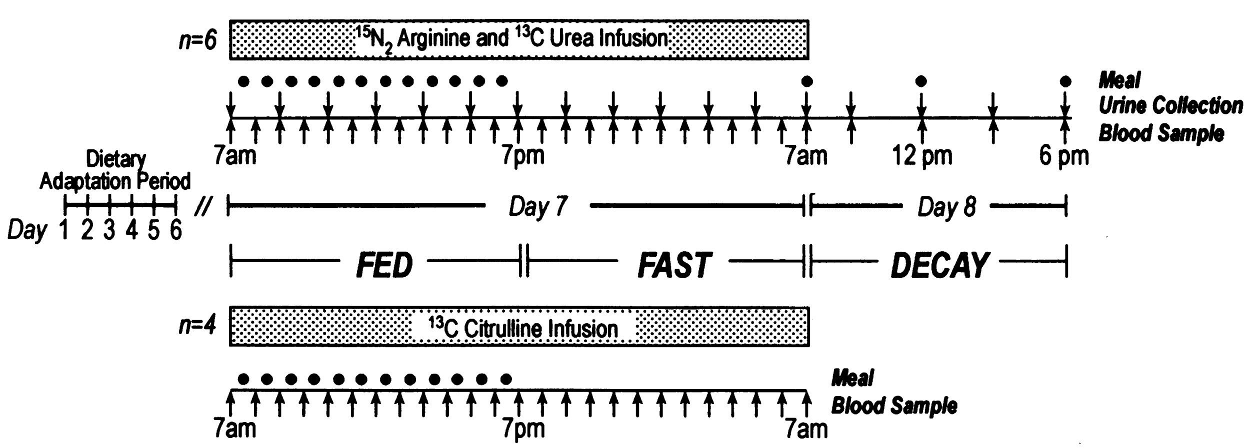

FIG. 1. Experimental design used to explore arginine–citrulline–NO relationships in healthy adults

[*guanidino*-$^{15}N_2$]arginine; I_{Arg} is the rate of infusion of labeled arginine. The expression $Q_{Arg}/(I_{Arg} + Q_{arg})$ is added to correct for the contribution made by the tracer arginine infusion to the $Q_{Arg \to Cit}$. Comparable calculations were made to estimate $Q_{Arg \to urea}$ and $Q_{Cit \to Arg}$.

The rate of urea production also was estimated according to the standard isotope dilution equation (20). Urea excretion was determined from the measured urinary urea nitrogen output, corrected for any variation in the total body urea pool, as follows:

$$\text{corrected urea excretion} = \text{total urea excretion} - \left[\frac{\text{plasma urea (start)} - \text{plasma urea (end)} \times \text{total body water}}{0.92}\right],$$

where total body water was estimated according to Watson *et al.* (26) and plasma urea N was measured at hourly intervals from 0 and 720 min for the fed and 780 and 1440 min for the fasted state.

The rate at which plasma arginine was used for whole body NO production $f(Q_{Arg \to NO_3^-})$ also was estimated using the urinary NO_3^- method as follows:

$$f(Q_{Arg \to NO_3^-}) = \frac{^{15}NO_3^- \text{output } (\mu\text{mol) per study period (35 hr) per subject} \times 1.67}{[^{15}N]\text{arginine tracer } (\mu\text{mol given in 24 hr)}},$$

where "corrected" $^{15}NO_3^-$ output is the measured urinary $^{15}NO_3^-$ excretion for the entire study period [24-hr tracer infusion and observed decay period (i.e., total output over 35 hr), multiplied by the factor 1.67, which accounts for under-recovery in urine of orally administered $^{15}NO_3^-$, which is about 60% (27)]. Thus, the rate of conversion of plasma arginine to NO_3^- ($Q_{Arg \to NO_3^-}$) was obtained from the following equation:

$$Q_{Arg \to NO_3^-} = Q_{Arg}\, 24\text{hr} \times f(Q_{Arg \to NO_3^-}),$$

where Q_{Arg} 24 hr is the plasma arginine flux (μmol per subject) for the entire 24-hr study period.

Evaluation of Data. Comparisons between amino acid kinetics for both metabolic periods (fed vs. fasted) and of different estimates of NO synthesis ($Q_{Arg \to Cit}$ vs. urinary NO_3^- output), were made using the Student's paired *t* test. Data are expressed as mean ± SD. A value of $P < 0.05$ was considered to be statistically significant. Least squares linear regression analysis was applied to the urinary ^{15}N abundance data to approximate the total urinary ^{15}N excretion rate.

RESULTS

The plasma isotopic abundances in [$^{15}N_2$]arginine, [^{13}C]urea, and the derived [^{15}N]citrulline and [$^{15}N_2$]urea, for the 35-hr study period, are shown in Fig. 2. The labeling of arginine and urea was higher during the fasted state ($P < 0.05$), as expected (14–16, 28), indicating a lower plasma flux and urea production rate during this metabolic state. Plateau ^{15}N enrichment for citrulline was achieved within approximately 5–6 hr and this was maintained until the end of the 24-hr tracer infusion; average abundance (mol fraction) was 0.0058 ± 0.002 and 0.0072 ± 0.001, respectively, for the fed and fasted states ($P < 0.05$), whereas the [$^{15}N_2$]urea plateau continued to rise, although slowly, throughout both the fasted and fed portions of the 24-hr tracer infusion study (mean = 0.0027 ± 0.0005 for fed; 0.0054 ± 0.0008 for fasted, $P < 0.05$). The plasma isotopic enrichment of [*ureido*-^{13}C]citrulline and the derived [^{13}C]arginine is shown in Fig. 3. There was no significant influence of metabolic state on the plasma abundance of [^{13}C]citrulline

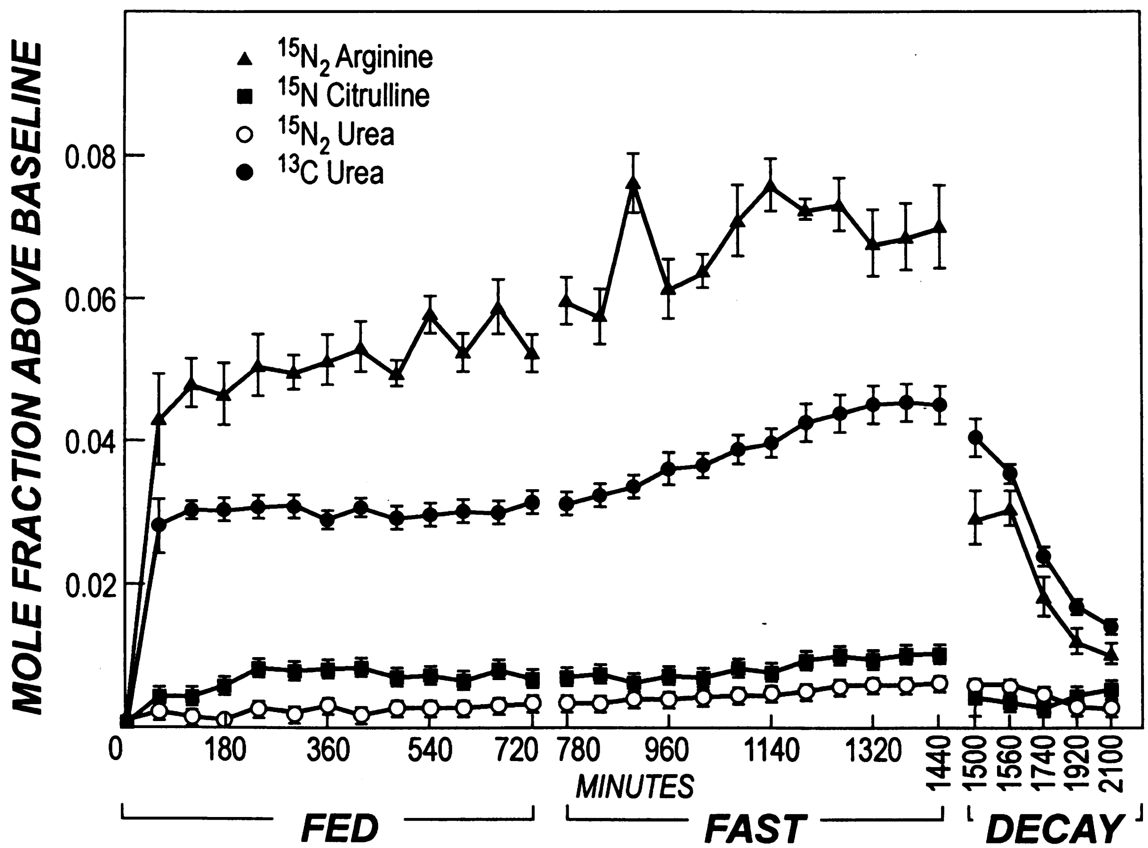

FIG. 2. Plasma isotopic abundance of [$^{15}N_2$]arginine, [^{13}C]urea, and derived metabolites ([^{15}N]citrulline and [$^{5}N_2$]urea) in six healthy adults. Isotope infusion was terminated at 1400 min. Error bar = ±1 SD.

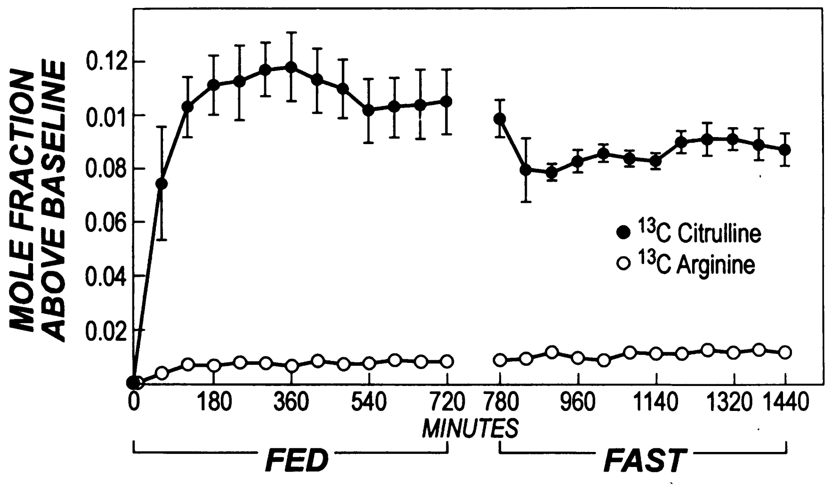

FIG. 3. Plasma isotopic abundance of [^{13}C]ureido citrulline and derived [^{13}C]arginine during a 24-hr continuous intravenous labeled citrulline infusion in four healthy men. Error bar = ±1 SD.

(0.106 ± 0.023 for fed vs. 0.089 ± 0.009 for fasted), but the [^{13}C]arginine abundance was lower ($P < 0.05$) during the fed state (0.009 ± 0.001 vs. 0.012 ± 0.002).

The ^{15}N enrichment of urinary nitrate during the fed, fasted, and decay periods throughout the 35-hr study period is shown in Fig. 4. An abundance of about 0.04 ± 0.001 atom percent excess was achieved by about 15 hr into the tracer study, and this was maintained until the tracers were stopped. By the end of the 11-hr "decay" period, urinary excretion of ^{15}N was still detectable. Using least squares linear regression analysis (r^2 = 0.97), we predicted that a very low rate of ^{15}N urinary excretion would have been reached in about 9 more hr, or by about hour 20 after terminating the [$^{15}N_2$]arginine tracer infusion. There was a relationship between urinary volume and nitrate output, with an average of 641 ± 135 μmol of NO_3^- in 2136 ± 715 ml of urine for fed and 344 ± 29 μmol in 739 ± 147 ml of urine for the post-absorptive state and, thus, higher nitrate excretion and urinary volume during the fed state (r^2 = 0.73; $P < 0.01$). During the fed state fluid intake (oral plus parenteral) was higher than for the post-absorptive state, when subjects received only the tracer infusion.

Plasma arginine flux (Table 1) averaged 82.3 ± 6.5 μmol·kg^{-1}·hr^{-1}, during the 24-hr period, whereas plasma citrulline fluxes averaged 9.5 ± 1.5 μmol·kg^{-1}·hr^{-1}. The rate of whole body NO synthesis (μmol·kg^{-1}·hr^{-1}) as estimated from the labeling of [^{15}N]citrulline was 0.91 ± 0.3 and 1.00 ± 0.2 for the fed and fasted periods, respectively, averaging 0.96 ± 0.1 μmol·kg^{-1}·hr^{-1} for the 24 hr. NO synthesis estimated by urinary total NO_3^- output was 1.23 ± 0.2 and 0.67 ± 0.05 μmol·kg^{-1}·hr^{-1} for fed and fasted states, respectively, with an average value of 0.95 ± 0.1 μmol·kg^{-1}·hr^{-1} for the 24-hr period. Hence, there was excellent agreement between both of these methods of estimation of whole body NO synthesis over the 24-hr period. The percentage of plasma arginine flux that

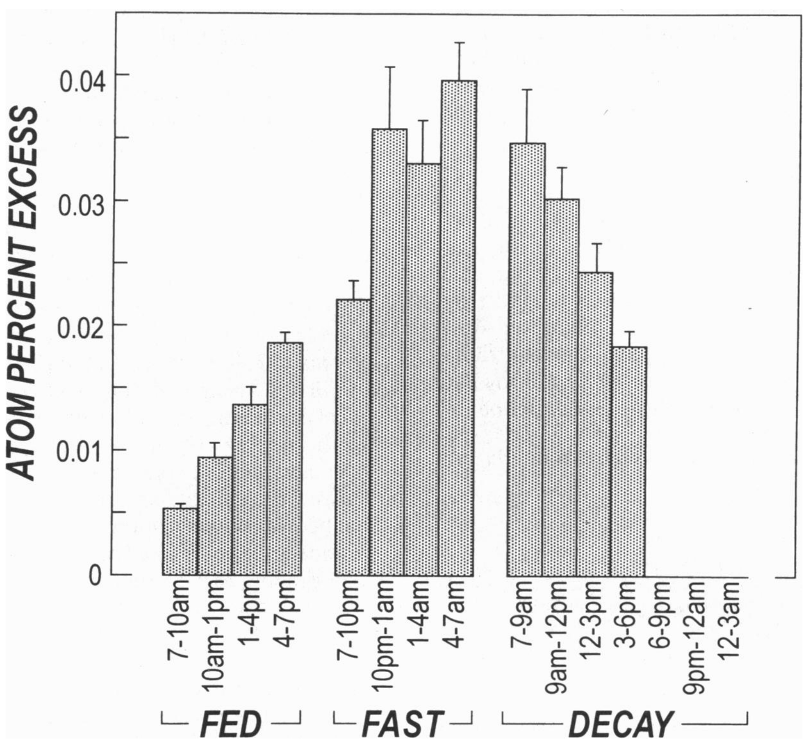

FIG. 4. Urinary ^{15}N enrichment atoms percent excess during fed, fasted, and decay periods following a 24-hr constant intravenous infusion with [$^{15}N_2$]arginine in six healthy adults. Error bar = +1 SD.

Table 1. Plasma arginine and citrulline fluxes, *de novo* arginine synthesis, and estimate of NO synthesis by ^{15}N-labeled plasma citrulline and by urinary nitrate output

	Value		
	Fed	Fasted	Average for 24 hr
Arginine flux (Q_{Arg})*	94.6 ± 5.8†	70.2 ± 7.8	82.3 ± 6.5
Citrulline flux (Q_{Cit})*	8.8 ± 2.0	10.3 ± 1.2	9.5 ± 1.5
De novo arginine synthesis ($Q_{Cit \rightarrow Arg}$)*	7.8 ± 1.9†	10.7 ± 1.1	9.2 ± 1.4
NO synthesis ($Q_{Arg \rightarrow Cit}$)*	0.91 ± 0.3	1.00 ± 0.2	0.96 ± 0.1
NO synthesis (NO_3^- output)*	1.23 ± 0.2†	0.67 ± 0.5‡	0.95 ± 0.1
% $Q_{Arg\ to\ Cit}$ (NO synthesis)			1.2 ±0.13

All values are mean ± STD.
*$\mu mol \cdot kg^{-1} \cdot hr^{-1}$.
†<0.05 fed vs. fasted.
‡$P < 0.05$ NO_3^- output vs. $Q_{Arg \rightarrow Cit}$.

was associated with NO synthesis (percent $Q_{Arg\ to\ Cit}$) was 1.2 ± 0.13 for these healthy subjects.

From the measured output of $^{15}NO_3$ and calculated rate of transfer of [^{15}N]arginine in plasma to $^{15}NO_3^-$ in urine, an estimation can be made of the contribution by arginine in plasma to total body NO synthesis. As summarized in Table 2, we conclude that about 54% of total NO synthesis is derived from arginine leaving the plasma compartment.

Urea kinetics over the 24-hr tracer study period are summarized in Table 3. Urea production was 239 ± 28 and excretion was 237 ± 28 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ during the fed state; both values were significantly higher than those for the fasting period. Intestinal urea hydrolysis was 65 $\mu mol \cdot kg \cdot hr^{-1}$ or 27% of production during the fasted state, and it was close to 0% during the 12-hr fed period. The rate of transfer of plasma arginine to urea was 11.5 ± 1.06 and 12.8 ± 1.35 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ for fed and fasted, respectively; the fraction of plasma arginine flux associated with urea formation, therefore, was 12.1% for fed and 18.2% for fasted states; about 5–6% of the urea produced is derived from plasma arginine.

DISCUSSION

We have determined the rate of whole body NO synthesis estimated by means of measurement of conversion of [*guanidino*-$^{15}N_2$]]arginine to [*ureido*-^{15}N]citrulline is equivalent to the estimated whole body NO synthesis rate as judged from determination of urinary total nitrate/nitrite excretion. Arginine within the plasma compartment appeared to contribute about 54% of the substrate used in daily whole body NO synthesis. These major observations will be discussed below.

Table 2. Metabolic fate of plasma arginine in relation to urinary nitrate output

Parameter	Value*
Corrected $^{15}NO_3^-$ excretion†	54.6 ± 7.5
$Q_{Arg \rightarrow NO_3^-}$‡	883.9 ± 127.3
% NO_3^- from plasma arginine§	54.4 ± 9.1

*Mean ± SD.
†Output (μmol per subject) over 35 hr corrected for an underrecovery factor of 1.67 (see *Materials and Methods*).
‡μmol of plasma arginine, per day per subject, used for NO synthesis.
§Percent daily.

Table 3. Urea kinetics in healthy subjects receiving an adequate arginine intake

	Value	
	Fed	Fasted
Urea production*	239 ± 28†	184 ± 21
Urea excretion*	237 ± 28†	120 ± 14
Urea hydrolysis*	0†	65
$Q_{Arg \rightarrow urea}$*	11.5 ± 1.06	12.8 ± 1.35
% plasma arginine to urea	12	18
% urea derived from plasma arginine	5	6

Mean ± STD.
*$\mu mol \cdot kg^{-1} \cdot hr^{-1}$.
†$P < 0.05$ fed vs. fasted.

However, it should be pointed out here that the model of Clarke and Bier (23) we have used is an exploratory paradigm for interrelating the precursor product relationships in terms of both mass and isotopic label transfer, during conversion of arginine's guanidino group into nitrate. It should be recognized that the number of identifiable intermediate transformations in the course of NO and nitrate formation may be far more complicated, by physiologic and temporal compartmentation, than the analogous pathways for the ultimate disposal of phenylalanine into CO_2 via tyrosine and *P*-hydroxyphenyl pyruvate. The apparent mismatch between the percent contribution of systemic arginine to urinary nitrate and the putative contribution of intracellular arginine to whole body NO synthesis determined from its conversion to citrulline from isotope kinetics, will need to be resolved by more rigorous modeling paradigms now under development for application in further studies. Furthermore, the value of 54% is still underestimated, because we did not collect urine until the $^{15}NO_3$ output had essentially ceased completely, which takes about 24 h after the tracer infusion is terminated.

The plasma arginine flux was decreased during the fasted state, reflecting the lack of entry of dietary arginine into the plasma pool. This finding is consistent with our previous studies (14–16, 27). The plasma [*ureido*-^{13}C]citrulline fluxes of 8.8 (fed) and 10.3 (fasted) $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ determined in this investigation, were similar to those previously reported by our group, although slightly lower for the fasted state (14, 28). Using a more sensitive evaluation of the mass spectrometric data in this investigation, as compared with that followed earlier, the labeling of [^{13}C]arginine from [^{13}C]citrulline was detected throughout the study period; this has allowed us to estimate the rate of *de novo* arginine synthesis from plasma citrulline. Thus, we find an average *de novo* arginine synthesis rate during the 24-hr study period of 9.2 ± 1.4 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$. This indicates that under the present conditions, about 11% of the plasma arginine flux is derived from the conversion of plasma citrulline, whereas the remaining plasma arginine flux originates from different sources, including tissue protein breakdown and diet. Lower estimates of *de novo* arginine synthesis were obtained with [$^{2}H_2$]arginine in a previous study (28), and this is explained by the recycling of the [$^{2}H_2$] label. However, a reliable level of plasma [^{13}C]arginine labeling had not been previously obtained (14). This may be due to the more refined and precise analytical methodology used in our current experiment (18). Furthermore, the present tracer study period was prolonged to 24 hr, in comparison with the shorter 3 hr fast; 5-hr fed tracer periods studied previously (14, 28), and this may also have given us a better basis for estimating [^{13}C]citrulline-to-arginine conversion.

Whole body NO synthesis rates by both the [^{15}N]citrulline and urinary NO_3^- approaches were estimated to be about 0.95 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ over a 24-hr period, when the urinary NO_3^- was corrected for underrecovery of NO_3^- in the urine, which we have taken to be about 60% (27). This factor was obtained previously as an average for nitrate excretion during combined fasted and fed states. Therefore, this may explain the differences observed between the estimates of NO synthesis rates during the separate fed and post-absorptive states when obtained with the ^{15}N citrulline method vs. the urinary nitrate method (Table 1). Furthermore, the difference in NO synthesis rates observed among fed and post-absorptive states with the urinary nitrate method, also might be explained by the variation in urine output and nitrate excretion among the fed and post-absorptive periods. The 24 hr average NO synthesis rate, however, gives similar values with both methods. The fraction of the whole-body arginine flux used for NO formation was 1.2% for our group of healthy subjects. Similarly, Becker and coworkers (29), using stable isotopic techniques in normal and burned rats, reported whole body NO synthesis to be less than 1% of arginine metabolism.

With respect to urea metabolism, we found that the rate of conversion of plasma arginine to urea was about 11.5–12.8 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ and did not differ significantly between fed and fasted states. These results indicate that over a period of 24 hr only 15% of arginine disappearance from plasma occurred via urea formation. It appears that urea formation occurs from intracellular sources of arginine that are not in rapid or extensive equilibrium with the plasma pool, as our previous tracer studies (15, 20) have revealed.

In summary, we have explored the rate of whole body NO synthesis in healthy subjects by two different approaches, both yielding average estimates of about 0.95 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ for the 24-hr period. *De novo* arginine synthesis from plasma citrulline over the same period accounts for about 11% of the plasma arginine flux, for the dietary conditions employed. This value may be influenced by the dietary intake level of nitrogen and of amino acids. It is clear from this study that the quantitative contribution of the plasma arginine compartment to NO synthesis is significant but to urea synthesis in healthy humans it is small. The extent and nature of the changes that occur in these metabolic relationships during various pathophysiologic conditions in humans, remain to be established. Our tracer model should be useful to investigators concerned with quantitative aspects of arginine–NO–urea interrelationships in different human population groups.

We thank the nursing and dietary staff at the Clinical Research Center (CRC)–Massachusetts Institute of Technology, M. Kenneway, and A. Sevaragh their involvement. This work was supported largely by National Institutes of Health Grant DK 15856 and in part by National Institutes of Health Grants RR80 (CRC Core Grant) and GM 02700 and the Shriners Hospitals for Crippled Children.

1. Barbul, A. (1986) *J. Parenter. Enteral Nutr.* **10,** 227–238.
2. Visek, W. J. (1986) *J. Nutr.* **116,** 36–46.
3. Moncada, S. & Higgs, A. (1993) *N. Engl. J. Med.* **329,** 2002–2012.
4. Nathan, C. (1992) *FASEB J.* **6,** 3051–3064.
5. Hibbs, J. B., Taintor, R. R., Vavrin, Z., Granger, D. L., Drapier, J. C., Amber, I. & Lancaster, J. (1990) in *Nitric Oxide from L-Arginine: A Bioregulatory System,* eds. Moncada, S. & Higgs, E. (Excerpta, New York), pp. 189–273.
6. Drexler, H., Zeiher, A. M., Meinzer, K. & Hanjorg, J. (1991) *Lancet* **338,** 1546–1549.
7. Creager, M. A., Gallagher, S. J., Girard, X. J., Coleman, S. M., Dzau, V. J. & Cooke, J. P. (1992) *J. Clin. Invest.* **90,** 1248–1253.
8. Moribawa, E., Moskowitz, M., Huang, Z., Yoshida, T., Irikura, K. & Dalkara, T. (1994) *Stroke* **25,** 429–435.
9. Alexander, J. W. & Gottschlich, M. M. (1990) *Crit. Care Med.* **18,** S149–S153.
10. Meyer, J., Traber, L. D., Nelson, S., Lentz, C. W., Nakazawa, H., Herndon, D., Noda, H. & Traber, D. L. (1992) *J. Appl. Physiol.* **73,** 324–328.
11. McCartney-Francis, N., Allen, J. B., Mizel, D. E., Albina, J. E., Xie, Q., Nathan, C. F. & Wahl, S. M. (1993) *J. Exp. Med.* **178,** 749–754.
12. Petros, A., Bennett, D. & Vallance, P. (1991) *Lancet* **338,** 1557–1558.
13. Castillo, L., DeRojas, T., Chapman, T., Tannenbaum, S. & Young, V. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 193–197.
14. Castillo, L., Sanchez, M., Vogt, J., Chapman, T., Ajami, A., de Rojas-Walker, T., Tannenbaum, S. R. & Young, V. R. (1995) *Am. J. Physiol.* **268,** E360–E367.
15. Beaumier, L., Castillo, L., Ajami, A. & Young, V. R. (1995) *Am. J. Physiol.* **269,** E884–E896.
16. Castillo, L., de Rojas-Walker, T., Yu, Y.-M., Sanchez, M., Chapman, T. E., Shannon, D., Tannenbaum, S., Burke, J. F. & Young, V. R. (1995) *Pediatr. Res.* **38,** 17–24.
17. Green, L. C., Ruiz de Luzuriaga, K., Wagner, D. A., Rand, W., Istfan, N., Young, V. R. & Tannenbaum, S. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7764–7768.
18. Rosenblatt, J., Chinkes, D., Wolfe, M. & Wolfe, R. R. (1992) *Am. J. Physiol.* **263,** E584–E596.
19. Castillo, L., Ajami, A., Branch, S., Chapman, T. E., Yu, Y.-M., Burke, J. F. & Young, V. R. (1994) *Metabolism* **43,** 114–122.
20. El-Khoury, A., Fukagawa, N., Sanchez, M., Tsay, R. H., Gleason, R. E., Chapman, T. E. & Young, V. R. (1994) *Am. J. Clin. Nutr.* **59,** 1000–1011.
21. Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, V. S. & Tannenbaum, S. R. (1982) *Anal. Biochem.* **126,** 131–138.
22. Fern, E. B., Garlick, P. J., McNurlan, M. H. & Waterlow, J. C. (1981) *Clin. Sci. (London)* **61,** 217–228.
23. Clarke, J. T. R. & Bier, D. M. (1982) *Metabolism* **31,** 999–1005.
24. Thompson, G. N., Pacy, P. J., Merritt, H., Ford, G. C., Read, M., Cheng, K. N. & Halliday, D. (1989) *Am. J. Physiol.* **238,** E473–E479.
25. Castillo, L., Sanchez, M., Chapman, T., Ajami, A., Burke, J. & Young, V. R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6393–6397.
26. Watson, P. E., Watson, I. D. & Batt, R. (1980) *Am. J. Clin. Nutr.* **33,** 27–39.
27. Wagner, D. A., Schultz, D. S., Deen, W. M., Young, V. R. & Tannenbaum, S. R. (1983) *Cancer Res.* **43,** 1921–1925.
28. Castillo, L., Chapman, T., Sanchez, M., Yu, Y.-M., Burke, J. F., Ajami, A. M., Vogt, J. & Young, V. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 7749–7753.
29. Becker, W. K., Shippee, R. Y., McManus, A. T., Mason, A. D. & Pruitt, B. A. (1993) *J. Trauma* **34,** 855–862.